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14. ABSTRACT
This Impact Award between the University of Pittsburgh, Stanford University and University of Wisconsin at Madison seeks to provide definitive clinical validation of low copy number transcript fusions as prognostic markers in clinically localized prostate cancer. The Stanford site is charged with providing formalin fixed paraffin embedded samples for independent validation of markers discovered at University of Pittsburgh. To that end, we have shipped 300 samples for assay testing and have accumulated over 600 FFPE blocks of radical prostatectomy specimens with detailed clinical follow-up. The assay results suggest that there are some artifacts, and University of Pittsburgh will be addressing these issues. Next year we will increase the number of samples accumulated to get to our goals. We will be sending specimens blinded to the Pittsburgh site and include controls for assay performance. We have agreed to allow the Stanford and Wisconsin sites to receive data from Pittsburgh to perform correlation with clinical data and correlation with outcomes at these sites.

15. SUBJECT TERMS
Prostate cancer, prognosis, gene fusions, fusion transcripts

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Annual Report - Stanford University
W81XWH-16-1-0542
PI: James D. Brooks

1. INTRODUCTION: The University of Pittsburgh site, University of Wisconsin site and Stanford site have previously published work demonstrating that, with very deep sequencing, we could identify gene fusions that predict with high sensitivity recurrence after radical prostatectomy (Am J Path **184:** 2857-2866, 2014). In this proposal, we seek to test whether the following fusions correlate with outcome: MAN2A1-FER, SLC45A2-AMACR, TRMT11-GRIK2, MTOR-TP53BP1, LRRC59-FLJ60017, CCNH-C5orf30, KDM4-AC011523.2, TMEM135-CCDC67. Furthermore, we propose to build a clinical model that includes some or all of the fusions to predict outcomes after surgery. The study design was to build a model on an initial set of samples from all institutions and validate on a second set of independent samples taken from the archival tissues available at each of the 3 sites.

2. KEYWORDS: Prostate cancer, prognosis, gene fusions, fusion transcripts

3. ACCOMPLISHMENTS: We have made significant progress toward the Stanford site goal of obtaining 1500 samples of radical prostatectomy specimens for validation of the performance of the fusion transcripts as prognostic markers.

What were the major goals of the project?

1. *Provide samples to University of Pittsburgh for prognostic model construction (Phase 1).*
2. *Accumulate additional samples at the Stanford site for definitive and independent validation (Phase 2)*

What was accomplished under these goals?

Task 1: *Provide samples to University of Pittsburgh for prognostic model construction (Phase 1).*

The Stanford site has provided its share of samples for model building and testing. The proposal stated we would build the model using a cohort of 600 samples from phase 1 that have at least 5 years clinical follow-up. The Stanford Site has met this metric by supplying 200 samples to the University of Pittsburgh.

Task 2: *Accumulate additional samples at the Stanford site for definitive and independent validation*

The Stanford site has now collected 600 radical prostatectomy samples that have associated long term clinical follow-up. These cases would be used for Phase 2 of the study in which the clinical model + fusions developed in Phase 1 will be validated for predicting outcome. Cores will be taken from those cases to be sent to the University of Pittsburgh over the next year for the grant. We are on track for collecting the 1500 cases over 3 years as stipulated in the SOW of the original proposal.

In this first round of model building (Phase 1), we have encountered problems with assay performance. The first set of samples sent by the Stanford site were sent blinded – i.e., without accompanying clinical data until the assays for the fusions were run. The Stanford PI (Brooks) then associated the outcomes for the assay with the clinical outcomes which he had available at the Stanford site. We had included in that initial set some internal controls – namely, paired normal prostate tissue samples for many of the prostate cancer cases.

The analysis by Dr. Brooks revealed that many fusion events were observed in the normal prostate tissues. It is conceivable that these events represent a field defect in the prostate, as has been described previously by Dr. Luo (transcript profiling published in JCO (15;22(14):2790-9, 2004) and Dr. Jarrard (Methylation published in J. Urol 189(6):2335-4, 2013). However, the more likely explanation is that the fusions detected in the normal tissues represent artifact.

However, there are several reasons to believe that the fusions detected are assay artifact. Most importantly, in our original publication in 2014 we did not detect fusions in any of the normal prostate tissues. Second, the fusions observed in the normal tissues were different from the fusions observed in the paired cancerous tissues, and there were many cases were fusions were observed in the normal tissues and not observed in the cancer from the same case. While Dr. Luo is moving ahead with testing whether the finding of the fusions in the cancer is correlated with outcome, the finding of fusions in the normal tissues that do not match those found in the cancer will confound any prediction based on the fusions. For example, if a fusion is observed in the normal tissue and not in the cancer, it could predict bad outcome, even though the tumor is not clonally related to the normal (since it lacks the fusion).

Dr. Brooks has raised these concerns at the most recent conference call of the team. This has led to considerable discussion among the group. Dr. Brooks feels that the assay is not optimized and that the detected fusions are PCR artifact. Dr. Luo is working hard to address those concerns. Suggested approaches from Dr. Brooks include retesting the samples from our original paper with his new optimized assay to make sure he obtains identical results as found in the first publication. Second, an internal control for PCR artifact could be included in the PCR assay such as primers for Green Fluorescent Protein (GFP) which should never be found in human tissues. Finally, Dr. Luo is assaying tissues (including normal tissues) for TMPRSS2:ERG fusions, since we know this fusion will occur in 50% of samples.

With the agreement of Dr. Nelson and Dr. Jarrard, Dr. Brooks and Dr. Jarrard will provide blinded samples to Dr. Luo's laboratory from now on for assay testing. The fusion calls by Dr. Luo's assay will be associated to clinical outcome only by the parent sites so that the assays are performed without knowledge of the clinical annotation. This is in accord with best practices of biomarker development and validation.

We have encouraged Dr. Luo to redesign his assays for detection of fusion transcripts. In addition, we have asked him to present data that demonstrate validation of the fusion transcripts, reproducibility of the assay etc. before we proceed with large scale model building validation. For his part, Dr. Brooks will include controls in all sample sets sent to University of Pittsburgh – replicate cases, samples of normal prostate tissue, and possibly some non-prostate tissues.

- **What opportunities for training and professional development has the project provided?**
  - *Nothing to report*

- **How were the results disseminated to communities of interest?**
  - *Nothing to report. We need confidence that the assay if functioning correctly before results can be disseminated.*
  -
- **What do you plan to do during the next reporting period to accomplish the goals?**
  - *Accumulate more samples. Send samples needed to Pittsburgh for assay development and validation*
  - *Critical scrutiny of assay performance.*

4. IMPACT:

- **What was the impact on the development of the principal discipline(s) of the project?**
  - *Since we have sent blinded samples, we have been able to detect potential issues with assay fidelity and performance. We will continue to uphold high standards of evidence for assay performance.*
- **What was the impact on other disciplines?**
  - *Nothing to report*
- **What was the impact on technology transfer?**
- *Nothing to report*
- **What was the impact on society beyond science and technology?**
- *Nothing to report*

2. **CHANGES/PROBLEMS:** *No changes*
   - **Actual or anticipated problems or delays and actions or plans to resolve them**
     - Prognostic Model development has been delayed because standards of assay performance have not been met. This will delay analysis of the validation samples until assay performance issues can be addressed by Dr. Luo at the University of Pittsburgh.
   - **Changes that had a significant impact on expenditures**
     - No changes
   - **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**
     - No deviations
   - **Significant changes in use or care of human subjects**
   - No changes
   - **Significant changes in use or care of vertebrate animals.**
   - Not applicable
   - **Significant changes in use of biohazards and/or select agents**
   - Not applicable
3. **PRODUCTS:**
   - **Publications, conference papers, and presentations**

     - **Journal publications.**
     - None

- **Books or other non-periodical, one-time publications.**
- None
- **Other publications, conference papers, and presentations.**
- None

- **Website(s) or other Internet site(s)**
  None
- **Technologies or techniques**
  None
- **Inventions, patent applications, and/or licenses**
  None
- **Other Products**
  None

4. **PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS**
   - **What individuals have worked on the project?**

Name:	*James Brooks, MD*
Project Role:	*PI*
Researcher Identifier (e.g. ORCID ID):	jdbrooks@stanford.edu
Nearest person month worked:	*1.20 calendar*
Contribution to Project:	*I have supervised the project at Stanford*
Funding Support:	This grant

Name:	*Rosalie Nolley*
Project Role:	*Pathology Technician*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	3.00 calendar
Contribution to Project:	*Pulled H & E slides* *Cored samples* *Shipped to U Pittsburgh* *Confirmed pathology under supervision of Dr. Brooks*
Funding Support:	This grant

Name:	*Michelle Ferrari*
Project Role:	*Research Nurse Coordinator*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	6.00 calendar
Contribution to Project:	*Pulled clinical data on all patients* *Constructed clinical databases* *Communicated blinded clinical data to Dr. Brooks who sends blinded data to Pittsburgh*

Funding Support:	This grant

Name:	*Kieu My Huynh*
Project Role:	*Research technician*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	5.00 calendar
Contribution to Project:	*Pulled H & E slides with Ms. Nolley* *Cored samples* *Shipped to U Pittsburgh* *Confirmed pathology under supervision of Dr. Brooks & Ms. Nolley*
Funding Support:	This grant

- **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**

ACTIVE
Title: IQGAP1 Scaffold-Kinase Interaction Blockade In Renal Cell Carcinoma: A Novel Biomarker And Therapeutic Strategy
Effort: 0.60 calendar
Supporting Agency: Department of the Army
Grants Officer: Wendy Baker
Performance Period: 09/15/2016-09/14/2019
Funding Amount: $122,015
Project Goals: The goal of this proposal is to test whether the scoffold protein IQGAP plays a significant goal in conventional renal cell carcinoma. Candidate signaling pathways associate with IQGAP will be interrogated. IQGAP will be assessed as a biomarker of disease recurrence. Strategies to block IQGAP function will be evaluated as therapeutic targets.
PI: Leppert, John
Role: Co-Investigator
Overlap: None.

Title: 68Ga Bombesin PET/MRI in Patients with Biochemically Recurrent Prostate Cancer and Noncontributory Conventional Imaging
Effort: 0.12 calendar
Supporting Agency: Department of Defense
Performance Period: 09/30/2016-09/29/2019
Funding Amount: $247,267
Project Goals: The goal of this proposal is to test the limits of detection of PET/MRI with a Bombesin tracer to localize recurrences in men after primary therapy for localized prostate cancer. The scans will be compared to conventional imaging.
Specific Aim: To compare the diagnostic performance of ^{68}Ga-RM2 PET/MRI to that of conventional imaging (CI) for detecting recurrent prostate cancer.
We hypothesize that:
1. At least 30% of patients will have one or more lesions detected on ^{68}Ga-RM2 PET/MRI.
2. The proportion of patients with detected lesions will be higher for ^{68}Ga-RM2 PET/MRI than for MR alone.

PI: Iagaru, Andrei
Role: Co-Investigator
Overlap: None.

Title: Metabolic imaging comparisons of patient-derived models of renal cell carcinoma
Effort: 0.12 calendar
Supporting Agency: NIH
Performance Period: 07/01/2017-06/30/2022
Funding Amount: $182,792
Project Goals: The goal is to identify a preclinical model that accurately reflects the metabolic phenotype of kidney cancer.
Specific Aims:
Aim 1. HP 13C imaging of RCC PDXs. Eight existing PDXs spanning a spectrum of RCC clinicopathology will be established as subrenal grafts in RAG2-/-γC-/- mice. The metabolic phenotype of each PDX will be characterized by HP **1**3C MR imaging and steady state metabolic profiling. Metabolic studies will be accompanied by: immunohistochemical (IHC) evaluation of RCC biomarkers, proliferation and apoptosis; short-tandem repeat (STR) analysis to confirm unique identity of each PDX; DNA sequencing of *VHL* and *MET* genes; transcriptomic profiling by RNA-Seq; and activity assays of enzymes involved in key metabolic pathways. The metabolic, genotypic and immunotypic phenotype of each PDX will be the "gold standard" for comparison of the PDX-derived TSCs, cell cultures, and cell culture-generated xenografts in subsequent aims.
Aim 2. HP 13C studies of RCC PDX-derived TSCs in a 3D tissue culture bioreactor. Each PDX will be processed to generate precision-cut, thin (300-μm) tissue slices. These slices will be cultured in a novel NMR- compatible bioreactor and HP **1**3C MR studies and steady state metabolic profiling will be performed. IHC, genetic, transcriptomic and enzymatic assays will be carried out as in Aim 1. Data obtained for TSCs will be compared to that for each original PDX to ascertain similarities and divergence.
Aim 3. HP 13C studies of RCC PDX-derived cell cultures in a 3D tissue culture bioreactor. Primary cell cultures will be established from each PDX and characterized by IHC, genetic analyses, transcriptomic profiling, and enzyme activities. The metabolic phenotype of each PDX-derived cell culture will be determined by HP **1**3C MR and steady state metabolic profiling. The metabolic, genotypic and immunotypic phenotype of each cell culture will be compared to that of its PDX of origin.
Aim 4. HP 13C imaging of RCC PDX cell culture-generated xenografts. Each PDX-derived cell culture will be implanted in mice to establish xenografts. The metabolic phenotype of each xenograft will be determined by HP **1**3C MR imaging and steady state metabolic profiling. Data from IHC, genetic, transcriptomic and enzymatic assays, along with metabolic data, will be compared to that from original PDXs.
Aim 5. Comparison of metabolic responses of PDX-derived models in response to glutaminase inhibition. TSCs, cell cultures, and cell culture-generated xenografts derived from a PDX sensitive to growth inhibition by the clinically relevant glutaminase inhibitor, CB-839, and from a PDX resistant to CB-839, will be evaluated for fidelity of responsiveness to CB-839, then changes in metabolism in response to CB-839 will be compared among the sensitive and resistant PDX-derived models by HP 13C imaging**.**
PI: Kurhanewicz, John
Role: Co-Investigator, Site Principal Investigator
Overlap: None.

<u>COMPLETED</u>

Title: Molecular signatures of LUTS-associated BPH
P20 DK103093
Effort: 2.40 calendar
Supporting Agency: NIDDK
Grants Officer: Deborah Hoshizaki
Performance Period: 07/01/2014-06/30/2017
Funding Amount: $317,037

Project Goals: The goal of this proposal is to use high throughput sequencing, in particular RNA-seq, to identify molecular genetic alterations in BPH tissues that are associated with LUTS. Our goal is to develop a molecular classification of BPH, define new pathways of etiology and possibly develop biomarkers associated with BPH and LUTS. We will also develop a culture system for growing immortalized epithelial cells derived from BPH tissues. In parallel, we will develop a training program centered on genomics and cell culturing methods to train new investigators to carry out research in benign urologic diseases
Specific Aim: The Scientific Research Project addresses the research goals of the Center with aims that will generate a new molecular classification of BPH and functionally examine candidate drivers within each subclass. These aims will involve sequence-based transcriptional profiling and the development of novel BPH cell culture models using conditionally reprogrammed cell (CRC) technology.
PI: Brooks, James
Role: Principal Investigator
Overlap: None.

Title: PASS-GHI collaboration
Effort: 0.12 calendar
Supporting Agency: The Fred Hutchinson Cancer Research Center / Genomic Health
Performance Period: 11/04/2014-12/31/2016
Funding Amount: $27,000
Project Goals: The goal of this proposal is to test the performance of a molecular signature called OncotypeDx Prostate for its ability to identify patients at the time of diagnosis who are at risk for reclassification while on active surveillance. Reclassification events are defined as significant increases in tumor volume or grade on follow-up biopsy. The goal is to evaluate the use of the test in risk stratifying men participating in a prospective active surveillance registry trial called the Prostate Active Surveillance Study (PASS).
PI: Brooks, James
Role: Site Principal Investigator
Overlap: None

- **What other organizations were involved as partners?**
- University of Wisconsin & University of Pittsburgh as per award structure.

5. **SPECIAL REPORTING REQUIREMENTS**
   - **COLLABORATIVE AWARDS:** Please see separate reports from the University of Pittsburgh and the University of Wisconsin.